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NIXON & VANDERHYE, PC
901 NORTH GLEBE ROAD, 11TH FLOOR
ARLINGTON, VA 22203

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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

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*Ex parte* JOHN ROBERT BIRCH, ROBERT CHARLES BORASTON,
and MARTYN SHAW

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Appeal 2011-005341
Application 10/501,777
Technology Center 1600

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Before ERIC GRIMES, FRANCISCO C. PRATS, and
JEFFREY N. FREDMAN, *Administrative Patent Judges*.

GRIMES, *Administrative Patent Judge*.

## DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of increasing glycosylation of a protein expressed by a human cell. The Examiner has rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

## STATEMENT OF THE CASE

The Specification discloses that "mammalian cell cultures are the preferred source of a number of important proteins for use in human and

animal medicine, especially those which are relatively large, complex and glycosylated" (Spec. 1: 9-11). The Specification discloses that "when glutamine is used as an energy substrate by cultured cells, ammonia is a catabolite produced, which is cytotoxic and can inhibit … glycosylation of proteins by its effect on pH within the Golgi of the cell" (*id.* at 1: 23-28). The Specification discloses "a glutamine-auxotrophic human cell … which has been transfected with a (first) exogenous DNA sequence encoding a protein…, and further with a (second) exogenous DNA sequence encoding a glutamine synthetase (GS) … said transfected cell [being] capable of producing said protein and capable of growing in a glutamine-free medium" (*id.* 2: 31 to 3: 5).

Claims 32-41 are on appeal. The claims have not been argued separately and therefore stand or fall together. 37 C.F.R. § 41.37(c)(1)(vii). Claim 32 is representative and reads as follows:

> 32. A method of increasing sialylation and/or N-glycan charge of a glycosylated protein expressed by a glutamine auxotrophic human cell and of extending the viability of said cell, said method comprising transfecting a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase to produce a transfected human cell and culturing said transfected human cell in a glutamine-free media such that said sialylation and/or N-glycan charge of said glycosylated protein is increased and the viability of said cell is extended.

*Issues*

The Examiner has rejected claims 32-41 under 35 U.S.C. § 103(a) as being obvious in view of Wilson[1] or Bebbington,[2] in view of Brandt,[3]

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[1] Wilson et al., WO 87/04462, published July 30, 1987.
[2] Bebbington et al., US 5,891,693, issued Apr. 6, 1999.

Schneider,[4] Gawlitzek,[5] Hermentin,[6] and Barsomian.[7] The Examiner finds that Bebbington discloses "mouse and rat lymphoid cell lines … transformed to glutamine independence by incorporating a gene encoding glutamine synthetase (GS) so that the cells can grow in glutamine-free medium" (Answer 4). The Examiner finds that Bebbington discloses that the cell also "contains a gene coding for a heterologous protein" such as tPA (*id.* at 4-5) and that Barsomian discloses that "tPA is a sialylated glycoprotein" (*id.* at 5).

The Examiner finds that Brandt discloses that it is advantageous to use human cells to produce human pharmaceutical proteins in order to obtain a "glycosylation pattern, especially a sialic acid profile[,] comparable to that of the naturally occurring … protein" (*id.*). The Examiner finds that both Gawlitzek and Schneider disclose that "ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine … [and] that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of recombinant GCSF" (*id.* at 6-7), and both suggest limiting glutamine in mammalian cell cultures (*id.* at 7). The Examiner finds that Hermentin discloses "a process for characterizing the glycosylation of glycoproteins" (*id.* at 8).

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[3] Brandt et al., US 6,395,484 B1, issued May 28, 2002.
[4] Schneider et al., *The importance of ammonia in mammalian cell culture*, 46 J. BIOTECHNOL. 161-185 (1996).
[5] Gawlitzek et al., *Ammonium Alters N-Glycan Structures of Recombinant TNFR-IgG: Degradative Versus Biosynthetic Mechanisms*, 68 BIOTECHNOL. BIOENG. 637-646 (2000).
[6] Hermentin et al., US 6,096,555, issued Aug. 1, 2000.
[7] Barsomian et al., US 5,238,821, issued Aug. 24, 1993.

The Examiner concludes that one of ordinary skill in the art would have been motivated to modify Bebbington's method to use a human host cell "to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins" (*id.* at 9). The Examiner further concludes that the

> ordinary skilled artisan seeking to increase the sialylation and/or N-glycan charge of a glycosylated protein, would have been motivated to express the desired protein in a cell without adding glutamine, since the prior art clearly teaches that ammonium, a byproduct of glutamine metabolism has a direct effect on the level of sialylation and/or N-glycan charge of a glycosylated protein.

(*Id.* at 11-12.)

Appellants contend that "[t]here is no suggestion in the cited art or reasonable expectation from the cited art that a glutamine auxotrophic cell line … could grow in a glutamine-free media ... in such a manner to increase sialylation and/or N-glycan charge and extend cell viability" (Appeal Br. 12). Appellants also argue that the method of claim 32 provides "unexpected benefits in increasing sialylation and/or N-glycan charge of a glycosylated protein and in allowing for a greater rate of protein synthesis and increased maximum product concentration" (*id.* at 23).

The issues presented are: Does the evidence of record support the Examiner's conclusion that the method of claim 32 would have been prima facie obvious in view of the cited references?

If so, have Appellants provided evidence of unexpected results that outweighs the evidence supporting the prima facie case of obviousness?

*Findings of Fact*

1. Schneider discloses that "[a]mmonia has been reported to be toxic and inhibitory for mammalian cell cultures for many years" (Schneider, abstract).

2. Schneider discloses that "high ammonia concentrations have been shown to perturb processing and secretion of proteins as well as glycosylation" (*id.* at 165).

3. Schneider discloses that the "main source of the ammonia which accumulates in cell cultures is glutamine" (*id.* at abstract).

4. Schneider discloses that one strategy to reduce ammonia formation in mammalian cell cultures is the adaptation of cells, including human cells, in glutamine-free medium (*id.* at 174-175).

5. Schneider discloses that ammonia has been shown to have deleterious effects on cell growth and viability (*id.* at 162-165, Table 1).

6. Gawlitzek discloses that "[a]mmonium … usually accumulates in mammalian cell cultures…. Ammonium is mainly a byproduct of glutamine metabolism and the thermal degradation of glutamine." (Gawlitzek 637.)

7. Gawlitzek discloses that "[n]umerous studies reported negative effects of elevated ammonium concentrations on cell growth and productivity of different cell lines" (*id.*).

8. Gawlitzek "used tumor necrosis factor receptor-immunoglobulin G (TNFR-IgG) as a model glycoprotein … to investigate the effect of ammonium on protein glycosylation in CHO cell cultures…. Ammonium decreased galactosylation and sialylation of TNFR-IgG N-glycans." (*Id.* at 638.)

9. Gawlitzek discloses that "[s]imilar mRNA levels (Fig. 6) and enzyme activities (Fig. 7) were found when comparing cells cultivated under low or high ammonium concentrations" (*id.* at 643).

10. Gawlitzek discloses that the results "strongly suggest that ammonium inhibits galactosylation and sialylation of TNFR-IgG N-glycans by pH-regulated mechanisms. We hypothesize that ammonium decreases α2,3-sialyltransferase and β1,4-galactosyltransferase activities by increasing the pH of the *trans*-Golgi compartment." (*Id.* at 644.)

11. Bebbington discloses that "many lymphoid cell lines … cannot be grown in vitro on media lacking in glutamine.… [I]t would be useful to be able to transform lymphoid cell lines to glutamine independence, since this may provide an advantageous method for selecting transformed cell lines." (Bebbington, col. 1, ll. 29-34.)

12. Bebbington discloses

> a method for transforming a lymphoid cell line to glutamine independence which comprises:
>
> transforming the lymphoid cell line with a vector containing an active glutamine synthetase (GS) gene;
>
> growing the transformed cell line on a medium containing glutamine; and
>
> continuing the growth of the transformed cell line on a medium in which the glutamine is progressively depleted or on a medium lacking glutamine.

(*Id.* at col. 1, ll. 51-60.)

13. Bebbington discloses that preferably the GS-encoding vector "also contains an active gene coding for a protein heterologous to the lymphoid cell line. Alternatively, the lymphoid cell line may be co-

transformed with a separate vector containing the active gene coding for the heterologous protein." (*Id.* at col. 2, ll. 25-30.)

14. Bebbington discloses that an example of a heterologous protein product is tissue plasminogen activator (tPA) (*id.* at col. 2, ll. 31-35).

15. Barsomian discloses that tPA is a sialylated glycoprotein (Barsomian, col. 2, ll. 49-51).

16. Brandt discloses a process for producing "human proteins … in a form suitable for producing a pharmaceutical composition" (Brandt, abstract).

17. Brandt discloses that a "human cell line is preferably used which synthesizes the target protein with a glycosylation pattern, especially regarding the number of the sialic acid moieties, comparable to that of the naturally occurring target protein" (*id.* at col. 3, ll. 25-30).

*Analysis*

Claim 32 is directed to a method that comprises transfecting a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase and culturing the cell in a glutamine-free media such that the sialylation and/or N-glycan charge of a glycosylated protein is increased and the viability of the cell is extended.

Schneider discloses that glutamine-associated ammonia accumulation inhibits mammalian cell cultures and also interferes with protein glycosylation. Schneider discloses that one strategy to reduce ammonia formation is to adapt cells, including human cells, to glutamine-free medium. Bebbington discloses transforming glutamine-auxotrophic cell lines with a selectable glutamine synthase gene so that the transformed cell

lines could be used for production of heterologous proteins such as tPA in glutamine-free media.

Brandt discloses the use of human cell lines for the production of pharmaceutical proteins with a glycosylation pattern comparable to that of the naturally occurring protein. Gawlitzek discloses that glutamine-associated ammonium inhibits galactosylation and sialylation of proteins by increasing the pH of the *trans*-Golgi compartment. In view of these disclosures, it would have been obvious to practice Bebbington's method of transforming glutamine-auxotrophic cells with a glutamine synthetase gene and culturing them on glutamine-free media with human cells, for the production of glycosylated human pharmaceutical proteins, in order to avoid the deleterious effects of glutamine-associated ammonium on protein sialylation and cell viability.

Appellants argue that "[t]here is no suggestion in the cited art or reasonable expectation from the cited art that a glutamine auxotrophic cell line … could grow in a glutamine-free media … in such a manner to increase sialylation and/or N-glycan charge and extend cell viability" (Appeal Br. 12). Appellants argue that the Examiner erred in relying on Gawlitzek to provide a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein by limiting glutamine and thereby ammonium in the media because Gawlitzek's Figure 7 "demonstrates that similar enzyme activities were found when comparing cells cultivated under low and high ammonium concentrations" (*id.* at 15). Appellants further argue that Gawlitzek's Figure 6 demonstrates that "similar mRNA levels of

the enzymes were found when comparing … cells cultivated under low or high ammonium concentrations" (*id.* at 17).

This argument is not persuasive. The results shown in Gawlitzek's Figures 6 and 7 demonstrate similar RNA and enzyme activities, respectively, with low and high ammonia concentrations (*see* FF 9). However, Gawlitzek concludes that its data show that ammonium decreases the activity of glycosylation enzymes by increasing the pH of the *trans*-Golgi compartment (*see* FF 10). The data shown in Gawlitzek's Figures 6 and 7 do not contradict this conclusion: Figure 6 shows mRNA levels, not enzyme activities; Figure 7, which does show enzyme activities, assayed those activities in cell *lysates* in order to determine whether ammonium has a direct effect on enzyme expression (*see* Gawlitzek 643: "To determine whether ammonia has a direct effect on enzyme expression, intracellular mRNA levels and enzyme activities of both glycosyltransferases were monitored over time in cells cultured under control and high ammonium conditions.").

Appellants argue that the claimed methods "provide unexpected benefits in increasing sialylation and/or N-glycan charge of a glycosylated protein and in allowing for a greater rate of protein synthesis and increased maximum product concentration" (Appeal Br. 22-23).

This argument is not persuasive. Unexpected results must be supported by factual evidence, and attorney argument is not evidence. *See In re Pearson*, 494 F.2d 1399, 1405 (CCPA 1974). Appellants have not provided any evidence that the Specification shows that the claimed method is unexpectedly superior to the closest prior art.

*Conclusions of Law*

The evidence of record supports the Examiner's conclusion that the method of claim 32 would have been prima facie obvious in view of the cited references. Appellants have not provided evidence of unexpected results that outweighs the evidence supporting the prima facie case of obviousness.

## SUMMARY

We affirm the rejection of claims 32-41 under 35 U.S.C. § 103(a).

## TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

## AFFIRMED

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